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J. LARS G. NILSSON

Studies of tocopherols and related chromanols

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Studies of tocopherols and related chromanols[1,2]

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In this survey the following papers, referred to by the Roman numerals listed below, will be discussed.

I. J. L. G. Nilsson, E. Redalieu, I. M. Nilsson and K. Folkers. On the protection against infarction by corn oil. *Acta Chem. Scand.* **22**, 97 (1968)

II. J. L. G. Nilsson, G. D. Daves, Jr. and K. Folkers. New tocopherol dimers. *Acta Chem. Scand.* **22**, 200 (1968)

III. J. L. G. Nilsson, G. D. Daves, Jr. and K. Folkers. The oxidative dimerization of α-, β-, γ-, and δ-tocopherols. *Acta Chem. Scand.* **22**, 207 (1968)

IV. J. L. G. Nilsson, H. Sievertsson and H. Selander. Tocopherols IV. Improved preparation of dimethylhydroquinones. *Acta Pharm. Suecica* **5**, 215 (1968)

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[1] Inaugural dissertation.

[2] Tocopherols XII. Paper XI in this series: J. L. G. Nilsson, H. Sievertsson and H. Selander. *Acta Pharm. Suecica* 5, 553 (1968).

V. J. L. G. Nilsson, H. Sievertsson and H. Selander. Synthesis of methyl substituted 6-hydroxychromans, model compounds of tocopherols. *Acta Chem. Scand.* **22**, 3160 (1968)

VI. J. L. G. Nilsson, H. Sievertsson and H. Selander. Infrared, ultraviolet and nuclear magnetic resonance spectra of methyl substituted 6-hydroxychromans, model compounds of tocopherols. *Acta Pharm. Suecica* 5, 553 (1968)

VII. J. L. G. Nilsson, H. Sievertsson and H. Selander. Tocopherols VI. Oxidation of methyl substituted 6-hydroxychromans with alkaline ferricyanide. *Acta Chem. Scand.* In press 1969

VIII. J. L. G. Nilsson, J.-O. Brånstad and H. Sievertsson. A quinone methide as a reaction intermediate in tocopherol oxidation. *Acta Pharm. Suecica* 5, 509 (1968)

IX. J. L. G. Nilsson, H. Sievertsson and H. Selander. On the preferential oxidative coupling at the 5- *versus* the 7-position in tocopherols and related 6-chromanols. *Acta Chem. Scand.* In press 1969

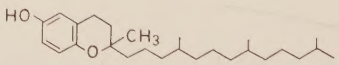
X. J. L. G. Nilsson and H. Sievertsson. Interconversion of α-tocopherol and its oxidation products. *Acta Pharm. Suecica* 5, 517 (1968)

Introduction

The discovery of oxidation products of tocopherols in fresh corn oil, described in the introductory Papers I—III led to an extensive investigation of tocopherol oxidation (Papers IV—X).

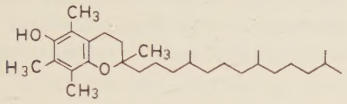
Research on vitamin E goes back to the work by Evans and Bishop [1] in 1922, when they discovered a factor essential for the prevention of a particular form of reproductive failure in female rats, characterized by the death and resorption of the foetus. The factor was traced to the lipid soluble components of certain foodstuffs, wheat germ oil being particularly active in preventing the resorption. Evans and his coworkers [2] succeeded in isolating the pure vitamin as a crystalline allophanate and they suggested the generic name tocopherol (from the Greek for "childbearing") [3] for the substance.

The vitamin E activity is not limited to one compound but is shared by a number of closely related derivatives of tocol (*1*). To date, eight such chromanols (*2*—*9*) have been isolated from natural sources. They belong

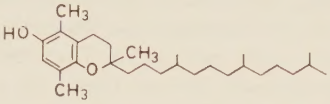


1 Tocol

to two series which differ in the degree of unsaturation of the isoprenoid side chain. The compounds with saturated side chain are referred to as α-, β-, γ- and δ-tocopherol (*2*—*5*), and those with unsaturated side chain as α-, β-, γ- and δ-tocotrienol (*6*—*9*) [4]. Of these compounds, α-tocopherol has the highest biological activity.



2 α-Tocopherol



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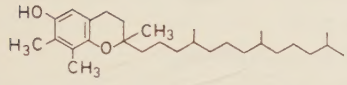
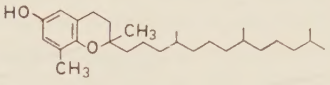
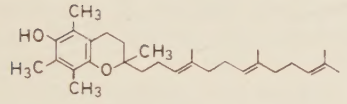


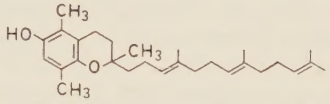
4 γ-Tocopherol



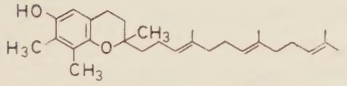
5 δ-Tocopherol



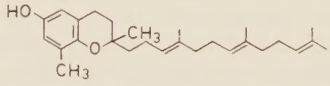
6 α-Tocotrienol



7 β-Tocotrienol



8 γ-Tocotrienol



9 δ-Tocotrienol

The tocopherols are synthesized by green plants. The biosynthetic sequence has recently been extensively studied. The tocotrienols are apparently precursors to the tocopherols [4, 5] and a full biosynthetic sequence from tyrosine has been recently published [6].

Animals and man obtain vitamin E from their diet, and it is then distributed throughout the tissues [7]. A wide variety of deficiency syndromes can be induced in experimental animals by feeding a diet low in tocopherols. The symptoms include the appearance in various species of muscular dystrophy, hepatic necrosis, infertility and abortion, encephalomalacia in birds and anaemia in primates [8]. The manifestations of vitamin E deficiency thus present a bewildering combination of symptoms, varying according to the species, age and diet of the experimental animal. It has been claimed [8—10] that the underlying factor common to all the tocopherols is their action as antioxidants, protecting the unsaturated lipids of the cells from oxidation. Whether the tocopherols possess other metabolic roles is a question to be elucidated in future research [11—13].

The primary cause for the cellular damage resulting from an inadequate functional level of the antioxidant in the cell is the formation of free radicals and peroxides by lipid oxidation. The mechanism of the tissue damage by these reactive species has been extensively studied and comprehensive reviews have been published [10, 14]. This lipid oxidation, which is primarily a free radical chain reaction [10], is effectively inhibited by antioxidants like the tocopherols. The chemical basis of vitamin E function is therefore its reaction with free radical intermediates and peroxides formed by lipid peroxidation. In this process, the tocopherols are oxidized to a variety of products, the study of which comprises part of this investigation.

Scope of the present investigation

This study was initiated during a search for the factor in corn oil which affords protection against infarction in rats maintained on an experimental diet (I). During chromatographic fractionation of the polar constituents of the oil, four phenolic tocopherol-like compounds were isolated. The structure elucidation (II) revealed that the compounds were dimers involving γ- and δ-tocopherol (*4* and *5*) and γ-tocotrienol (*8*). The identification was partly carried out by chromatographic and spectral comparison with synthetic tocopherol dimers prepared by oxidation of the appropriate tocopherols with *p*-benzoquinone (III). Dimerization of the four tocopherols (*2*—*5*) was therefore undertaken, and preparation of all the possible "mixed" dimers by cross coupling of two different tocopherol molecules was also attempted. These experiments revealed that the type of oxidation products formed from the tocol derivatives was dependant on the substitution pattern in the chroman moiety. The results also indicated a strong preference for reaction at the 5-position in the chroman moiety. This was particularly apparent in the oxidation of β-tocopherol (*3*) where benzylic type coupling *via* the 5-methyl group was preferred to the usual reaction pattern, in this case coupling at the unsubstituted 7-position, *ortho* to the hydroxyl group.

As a consequence of the results obtained, we were interested in establishing the structural requirements of the chromanol necessary for formation of the various types oxidation products. The proposed experiments would also reveal whether the observed high reactivity of position 5 was due to substituents in the aromatic ring or whether it was an intrinsic property of the bicyclic system itself. The data thus accumulated might then possibly lead to a mechanistic explanation of the phenomena and to a better understanding of the biological function of vitamin E.

For the proposed investigation we decided to use model compounds of the tocopherols. The natural tocopherols *2*—*5* all have an isoprenoid side chain in position 2, hence these compounds occur as oils at room temperature. For most studies of reactions of the chroman moiety, model compounds can be used where the isoprenoid side chain is replaced by a

methyl group. The synthesis of these compounds is described in Papers IV and V. Since structure elucidation of the oxidation products are largely based on spectral data, the spectra of the model compounds were extensively studied (VI). The model compounds were oxidized with alkaline ferricyanide (VII and VIII) or benzoquinone (IX) to give the appropriate dimers or trimers. Paper VIII describes a detailed study of the mechanism for formation of dimers and trimers from α-tocopherol. Finally the interconversion of α-tocopherol with its oxidation products was studied using ^{14}C-labelled compounds (X).

Corn oil and infarction

Cardiovascular and renal diseases accounted for 50 % of all deaths in the United States in 1963 [15]. Part of this disease complex is arteriosclerosis, which in itself represents a series of complicated pathological changes of the arterial wall. A serious complication to this is the formation of thrombi, which tend to develop in conjunction with the arteriosclerotic changes of the coronary arterial wall, thus causing a heart infarction. The result is the death of a group of myocardial cells through lack of oxygen, and if the impairment of the heart is severe, death of the individual follows.

A condition similar to human arteriosclerosis was produced in rabbits already in 1913 [16] by feeding the animals a cholesterol-rich diet. Many investigators could confirm this finding, but reports of infarctions in the experimental animals were very few. However, in a series of papers starting in 1957, Hartroft and coworkers [17] have described experimentally produced infarctions brought about by combining the previously used atherogenic cholesterol-rich diet with 40 % of fat. When this diet was fed to rats, myocardial infarctions occurred regularly. When corn oil was used in the diet instead of saturated fat, the rats were protected against infarction.

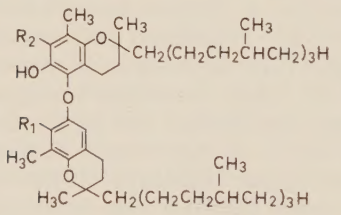
Paper I is the first report from a study on the biochemical basis for the protective effect of corn oil against infarction. During this work four phenolic compounds, similar to but less polar than γ-tocopherol, were isolated from corn oil. This discovery initiated the present study of tocopherol oxidation.

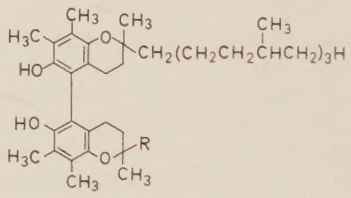
Isolation and structural elucidation of tocopherol dimers isolated from corn oil

Most studies on the constituents of vegetabilic oils have been carried out after saponification of the glycerol esters of the fatty acids. Since such treatment might well destroy the factor in corn oil responsible for the protection against infarction, a milder method had to be used. The fractionation described in Paper I was intended to afford mainly the polar constituents of corn oil. The neutral, non-polar lipids were removed from the more polar acidic components by passing a hexane solution of the fresh corn oil through a column of aluminium oxide. This retained the

more polar acidic materials such as phenols and acids, while the fatty acid esters were eluted (I). The retained material was then removed from the column with ether-methanol (3: 1), whereafter it was fractionated on a silica gel column using ether-hexane mixtures of increasing polarity as solvent. Tocopherol-like substances were detected in the early fractions and were purified by preparative thin layer chromatography (TLC) as described (II). Prior to this work, only few dimers and trimers of tocopherols have been isolated from natural sources. Draper *et al.* have isolated a dimer [18] and a trimer [19] of α-tocopherol from rat tissue and McHale and Green have isolated a dimer of γ-tocopherol, 5-(γ-tocopheryloxy)-γ-tocopherol[1] (*10*), from cotton seed oil deodorizer scum [20]. No other tocopherol dimers or trimers from natural sources had been reported when this study started, but several tocopherol oxidation products of other types have been isolated and characterized [11].

The tocopherol-like substances isolated from corn oil were identified as dimers mainly by mass spectroscopy. The exact structures were determined and were identified by analysis of their IR-, UV- and NMR-spectra as 5-(γ-tocopheryloxy)-γ-tocopherol (*10*). 5-(δ-tocopheryloxy)-γ-tocopherol (*11*), 5-(γ-tocotrienyl)-γ-tocopherol (*13*) and 5-(γ-tocopheryl)-γ-tocopherol (*14*). The structures were confirmed by comparison with authentic samples (II) prepared synthetically.





10 $R_1 = R_2 = CH_3$

11 $R_1 = H$; $R_2 = CH_3$

12 $R_1 = R_2 = H$

13 $R = -CH_2(CH_2CH{=}C(CH_3){-}CH_2)_3H$

14 $R = -CH_2(CH_2CH_2CH(CH_3)CH_2)_3H$

The mass spectra of the dimers deserve some comment. They all exhibit a peak corresponding to the dimer and also an intense peak corresponding to the monomer. The dimer from γ-tocopherol gives characteristic peaks at m/e 191 and 151, recently confirmed by Mayer *et al.* [21]. The nature of the process giving rise to the peak corresponding to the monomer is not fully understood, but may be due to an intramolecular hydride transfer and loss of a mesomeric phenoxy radical, a process related to the fragmentation of other aromatic ethers [22].

[1] The Chemical Abstracts name for this compound is 2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-5-[[2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-6-chromanyl]oxy]-6-chromanol.

Synthesis of the isolated dimers and of related tocopherol adducts

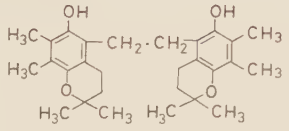
The need of synthetic tocopherol dimers for structural comparisons with the dimers isolated from corn oil induced us to undertake a study of the oxidative dimerization of α-, β-, γ- and δ-tocopherol (*2*—*5*) or of their model compounds where the isoprenoid side chain of the natural tocopherol had been replaced by a methyl group. Since two of the isolated dimers, compounds *11* and *13*, were formed through combination of two different tocol derivatives, the study also included the formation of "mixed" dimers.

Oxidation of α-tocopherol (*2*) has previously been extensively studied [11, 23, 24] and a variety of products including dimers and trimers have been described. A dimer of γ-tocopherol (*4*) was prepared by McHale and Green [20] and was shown to have structure *10*; no adducts of β-tocopherol (*3*) and δ-tocopherol (*5*) or "mixed" tocopherol dimers were known when this study was initiated.

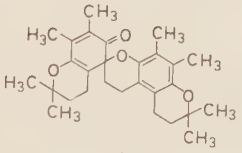
The oxidations were carried out by heating the appropriate tocopherol, or a binary mixture of two tocopherols with one equivalent of *p*-benzoquinone in benzene. The products were then isolated by preparative TLC and their structures were determined by analysis of their IR-, UV- and NMR-spectra.

From the oxidation of the α-tocopherol model compound, two dimers (*15* and *16*) and one trimer (*17 a*) were isolated. These compounds are all formed by reactions involving the methyl group in position 5.

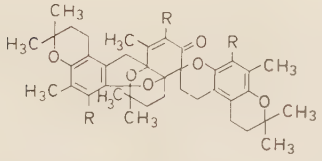
The trimer *17 a* was isolated as the main product (24 %) while the dimers *15* and *16* were obtained in smaller yields. All the three compounds have previously been described [23, 25, 26]. The spirodimer *16* is formed in high yield when the α-model is oxidized with alkaline ferricyanide [25]. Oxidation of γ- and δ-tocopherol gave dimers by carbon-oxygen and carbon-carbon coupling at the 5-position in the chroman



15



16

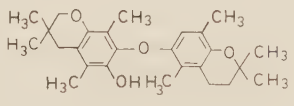


17a R = CH_3

17b R = H

moiety. Thus γ-tocopherol gave two dimers (*10* and *14*) identical in all respects with two of the dimers isolated from corn oil (I and II), while δ-tocopherol gave one diphenylether type dimer assigned structure *12*. The oxidative coupling of δ-tocopherol at position 5 to form *12* was assumed by reason of the preference for reaction at that position observed in α- and β-tocopherol. Later investigations showed that this assumption was correct (IX).

Oxidation of the β-tocopherol model compound (*23*) afforded three products. The main product was the trimer *17 b* analogous to the trimer *17 a* from the α-model. Two minor products, *18* and *19*, analogous to the dimers *10* and *14*, respectively, from γ-tocopherol were also isolated. The dimers *18* and *19* are formed by coupling at the unsubstituted 7-position of the chroman moiety. It is noteworthy that coupling products *via* the 7-position were not detected when the β-model was oxidized with benzoquinone at room temperature (IX). The results indicate that β-tocopherol reacts more easily *via* a benzylic type coupling (to give the trimer *17 b*) than by ordinary phenolic coupling at the unsubstituted 7-position *ortho* to the hydroxyl group.



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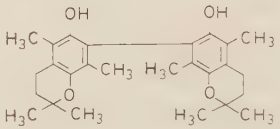
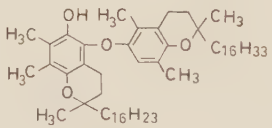


19

Formation of "mixed" dimers was studied by oxidizing all the possible binary mixtures of α-, β-, γ- and δ-tocopherol with benzoquinone. As described in Paper III, "mixed" dimers were isolated only when γ-tocopherol comprised one component of the binary mixture. The new compounds were identified as the βγ-dimer *20* and the γδ-dimer *21*; no other mixed dimers could be detected.



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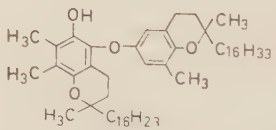


21

Analysis of the NMR-spectra of *20* and *21* revealed that both dimers were formed by attack of a β- or a δ-tocopheryloxy radical on γ-tocopherol. The alternative compounds, formed by attack of a γ-tocopheryloxy radical on β- or δ-tocopherol, were not detected. This indicates a higher reactivity of the 5-position in γ-tocopherol than of the unsubstituted positions *ortho* to the hydroxyl group in β- and δ-tocopherol.

Based on the results so far obtained and on available literature data (reveiwed in Paper III), a possible mechanism for the formation of the

tocopherol oxidation products was formulated (III). This mechanism has been reinvestigated and will be discussed later in this survey.

The results described in the three introductory papers (I—III) gave rise to the questions formulated below, the answers to which are of importance for the understanding of the chemistry of the bicyclic chroman structure and of the function of vitamin E:

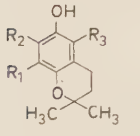
1) What causes the perferential reactivity of the 5-position in the 6-chromanols?
2) What are the structural requirements of the chromanols necessary for the formation of adducts like *15*, *16* and *17*, where one aromatic methyl group is involved in the reaction, or of dimers like *10* and *14*, formed by usual carbon-oxygen or carbon-carbon coupling?
3) Is the mechanism suggested in Paper III for formation of the spirodimer *16* correct?
4) α-Tocopherol forms the same oxidation products when oxidized with alkaline ferricyanide [23, 25, 26] or with benzoquinone (III). What products are formed from other tocol derivatives upon oxidation with alkaline ferricyanide?

Synthetic procedures

In the oxidation reactions studied here, model compounds of the tocopherols give products analogous to those formed from the natural tocopherols (III). Model compounds were therefore used in the following experiments as this enabled us to work with crystalline compounds of low molecular weight. The model compounds *22*—*29* were prepared as described below.

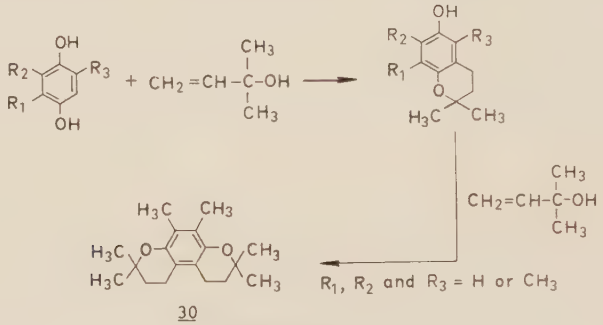
α-Tocopherol model compound (*22*) has been extensively used in the study of α-tocopherol chemistry (Paper V and references cited there). It was first prepared by Smith *et al.* [27] by condensing trimethylhydroquinone with isoprene in acetic acid, using zinc chloride as catalyst. By this method the pure α-model is obtained in good yield. However, when the same method was attempted in the preparation of tocol models with two or one aromatic methyl group (*23*—*28*) several isomers were formed and the products could be separated only after extensive chromatography.

1,1-Dimethylallyl alcohol was found to be useful for the preparation of the dimethyltocol models *23*—*25* and the tocol model (*29*). The alcohol



	R1	R2	R3	
22	CH_3	CH_3	CH_3	5,7,8-trimethyltocol model; α-model
23	CH_3	H	CH_3	5,8-dimethyltocol model; β-model
24	CH_3	CH_3	H	7,8-dimethyltocol model; γ-model
25	H	CH_3	CH_3	5,7-dimethyltocol model
26	CH_3	H	H	8-methyltocol model; δ-model
27	H	CH_3	H	7-methyltocol model
28	H	H	CH_3	5-methyltocol model
29	H	H	H	tocol model

and the appropriate hydroquinone were refluxed in formic acid and after removal of the acid the chromanol was isolated by a simple chromatographic procedure (V) (Scheme 1). Double chromans like *30*, formed from one molecule of the hydroquinone and two molecules of the allyl alcohol, were isolated as by-products. The dimethyl hydroquinones used were prepared as shown in Scheme 2. Small quantities of the hydroquinones can be prepared in very good yield by direct oxidation of the appropriate phenol with Fremy's salt [28] and subsequent reduction of the formed quinone. However, since the methods described in the literature [29] for preparation of the desired hydroquinones in large quantities (100 grams or more) did not give satisfactory yields, we modified these methods (IV) and obtained overall yields of 60—80 % of reasonably pure hydroquinones.



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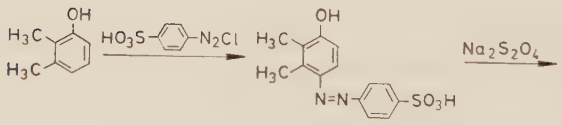
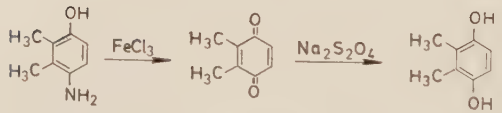
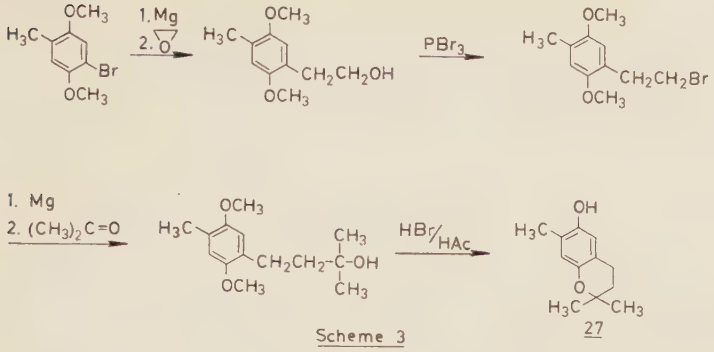
Scheme 1





Scheme 2

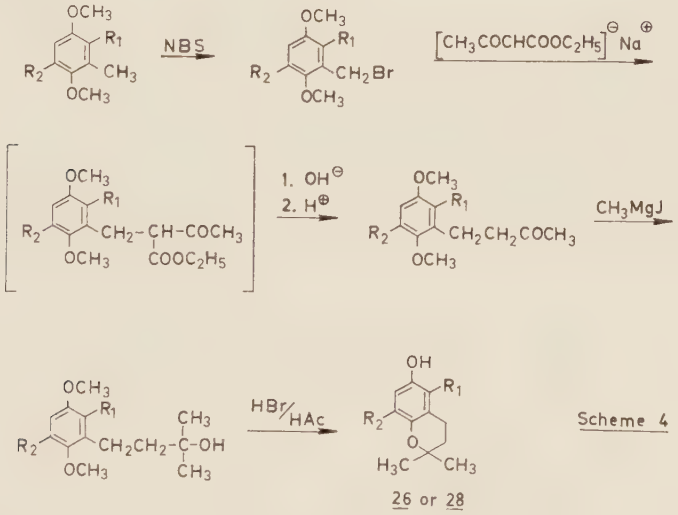
The model compounds with one aromatic methyl group (*26*—*28*) were prepared by methods that permitted an unequivocal structural assignment of the final products (V). Attempted preparation by condensation of toluhydroquinone with 1,1-dimethylallyl alcohol in formic acid gave a mixture of the model compounds of 5-, 7- and 8-methyltocol in the ratio of 1 : 2.6 : 1.5, as shown by gas chromatographic analysis of the reaction mixture. The separation of the compounds on a preparative scale was considered unfeasible and other methods for their preparation were therefore used.



Scheme 3

7-Methyltocol model (*27*) was prepared as shown in Scheme 3. This route was considered to be the most convenient for the preparation of *27* since the starting material, 5-bromotoluhydroquinone, is readily available.

Model compounds of 5- and 8-methyltocol (*28* and *26*) were prepared as shown in Scheme 4. After side chain bromination of the dimethyl hydroquinone dimethylether with N-bromosuccinimide, a ketone function was introduced through an acetoacetic ester synthesis. The ketone was reacted with methyl magnesium iodide and the resulting tertiary alcohol was converted to the chromanol by ether cleavage and cyclization using HBr in acetic acid.



Scheme 4

26 $R_1 = H$; $R_2 = CH_3$ 8-Methyltocol model; δ-model

28 $R_1 = CH_3$; $R_2 = H$ 5-Methyltocol model

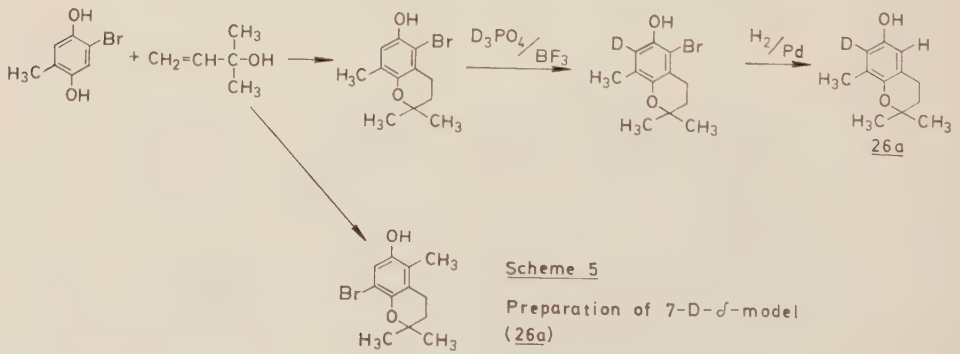
The procedures used for the synthesis of the 5-, 7- and 8-methyl tocol models (*26*—*28*) afforded each chromanol free from contamination with any of the other isomers. This purity is difficult to achieve if a preparation procedure is used that gives a mixture of isomers.

Spectral properties of the model compounds

IR-, UV- NMR-spectra of the methyl substituted chromanols *22* —*29* are described in Paper VI. The IR-spectra show six bands common for all the studied tocol derivatives in the region 850—1300 cm^{-1}. The bands occur at 1250—1240, 1225—1185, 1170—1160, 1125, 925—920 and 900—895 cm^{-1}. Most of these bands are strong and are well suited for the identification of chromanols of this type. Absorption bands in the region 950—1100 cm^{-1} seem to be due to aromatic methyl groups, since the tocol model *29*, which has no such groups, is the only one of the studied compounds that does not absorb in this region.

The UV-spectra are very similar to those reported for the natural tocopherols [30], and they all show absorption maxima around 300 mμ.

NMR-spectra of tocopherols have been published by Kofler *et al.* [30]. The main object of our NMR-study was to determine the chemical shifts of the 5- and 7-protons in δ-tocopherol. These protons give rise to two unsymmetrical doublets centered at $\delta = 6.20$ and 6.36 ppm, where each doublet represents one proton. To determine which of the two doublets represents the proton in position 5 or 7, we prepared the 7-D-δ-tocopherol model compound (*26 a*) and recorded its NMR-spectrum. This compound has only one aromatic proton whose band appears as a singlet at 6.20 ppm, representing the proton in position 5. The doublet centered at 6.20 ppm in the spectrum of the non-deuterated δ-model (*26*) is thus due to the 5-proton and the doublet at 6.36 ppm is consequently due to the proton in the 7-position. This knowledge permitted the elucidation of the structure of the dimer formed from the δ-model upon oxidation with benzoquinone (IX). The preparation of the 7-D-δ-tocopherol model (*26 a*) is described in Paper VI and was carried out as shown in Scheme 5.



Scheme 5

Preparation of 7-D-δ-model (26a)

Oxidation studies

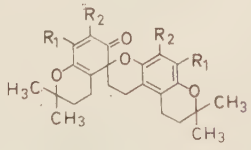
The experiments described below were designed to provide answers to the questions outlined above. The oxidation agents used in these studies were alkaline ferricyanide and benzoquinone. The former agent is widely used in studies of oxidative coupling of phenols and the mechanism of its action has been elucidated [31]. The initial oxidation step involves formation of a mesomeric phenoxy radical, which then can undergo coupling reactions to various products whose structures depend on the chemical reactivity of the aryloxy radical. The enzymatic oxidation of phenols in nature also involves formation of an aryloxy radical in the initial step [32], and alkaline ferricyanide oxidation of phenols can therefore be expected to simulate enzymatic oxidative coupling.

p-Benzoquinone, which is the other oxidizing agent used in this study, was found to produce the same dimers from γ- and δ-tocopherol as were isolated from corn oil (I—III). The isolated dimers were presumably formed by atmospheric oxidation when the tocopherols acted as antioxidants in the oil. Thus oxidation of phenols with benzoquinone may resemble atmospheric oxidation, which also is an important process in nature.

Oxidation with alkaline ferricyanide

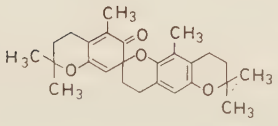
Oxidation of α-tocopherol (*2*) [18, 33] or of its model compound *22* [23, 25, 26, 34, 35] with alkaline ferricyanide has been studied by several workers. The reaction is carried out in a two-phase system, and the main product, formed in good yield, is the yellow spiro dimer *16*. The other minor coupling products isolated [25] may be formed in a secondary reaction as indicated in Papers VIII and X.

Oxidation of the β-model (*23*) and of the 5,7-dimethyltocol model (*25*) yielded spiro dimers analogous to the α-spiro dimer *16*. Spectral and chemical evidence presented in Paper VII are consistent with the structure *30 a* for the β-model spiro dimer and *30 b* for the dimer from the 5,7-dimethyltocol model. 5,7-Dimethyltocol model could theoretically give rise to three different spiro dimers (*30 b*, *31* and *32*). The dimer *30 b* would be formed by a reaction involving the 5-methyl group, *31* after



30a $R_1 = CH_3$; $R_2 = H$

30b $R_1 = H$; $R_2 = CH_3$



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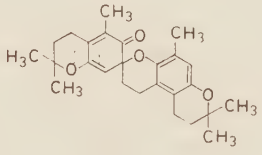

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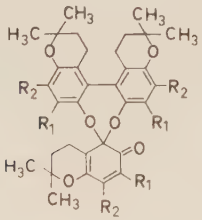
coupling of the 7-methyl groups and *32* by reactions involving the 5-methyl group in one molecule and the 7-methyl in the other.

Since the spiro dimer obtained was chromatographically pure and had a sharp melting point, structure *32* was excluded. If formation of *32* had occurred in the oxidation, a mixture of *30 b*, *31* and *32* would probably have been the result. By analysis of the NMR-spectrum, structure *31* could be excluded. The spectrum showed a small coupling ($J \sim 1$ cps) between the vinylic methyl group and a vinylic proton, and such a coupling can only occur in compound *30 b*.

Oxidation of the β-model (*23*) and of the 5,7-dimethyltocol model (*25*) shows that spiro dimer formation occurs when there is a 5-methyl group in the chromanol, and that this methyl group only and not a 7-methyl group is involved in the reaction.

Oxidation of the 5-methyltocol model (*28*) would also be expected to give a spiro dimer. However, more complicated polymeric products were isolated, and their structures were not studied. The polymeric products may have been formed by decomposition of an initially formed spiro dimer. It was observed that the stability of the spiro compounds described in this survey increased with the number of aromatic methyl groups present in the oxidized tocol model. Consequently, a spiro dimer from 5-methyl tocol model would probably decompose under the conditions used.

Oxidation of tocol models unsubstituted at position 5 (*24*, *26*, *27*, *29*) gave the spiroketal trimers *33*—*36*, formed by coupling at the 5-position of the aromatic ring. Thus the same reaction selectivity observed in the oxidation of *22*, *23* and *25* was exhibited also here. By forming the spiroketal trimers *33*—*36* the tocol models behave analogously to other alkyl substituted p-alkoxyphenols. Hewgill *et al.* [37] have demonstrated that formation of spiroketal trimers from such phenols on oxidation with alkaline ferricyanide is a very general reaction pattern.



	R_1	R_2	Oxidation product from
33	CH_3	CH_3	γ-model (24)
34	H	CH_3	δ-model (26)
35	CH_3	H	7-methyltocol model (27)
36	H	H	tocol model (29)

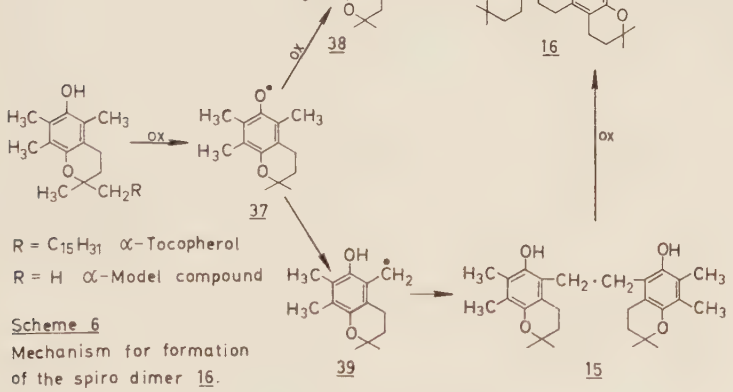
The spiroketal trimers of the tocols are very unstable and acid sensitive. When chromatography on silica gel or aluminum oxide was attempted, the originally yellow compounds were converted to green and red coloured materials and were therefore difficult to purify. The structures of the trimers are assigned on the basis of their IR-, UV- and NMR-spectra (XII).

Oxidation of the tocol models *22*–*29* with alkaline ferricyanide thus gives two different types of products, namely spiro dimers or spiroketal trimers. When a 5-methyl group is present as in *22*, *23* and *25*, spiro

dimers (*16*, *30 a* and *30 b*)are formed through reactions involving the 5-methyl group. When the chromanol has an unsubstituted 5-position, oxidation gives rise to spiroketal trimers (*33*—*36*). This demonstrates that the 5-methyl group or the 5-position in these chromanols are the favoured sites of reaction. Reaction *via* the 5-methyl group is preferred even in the case of the β-model (*23*) which has an unsubstituted 7-position *ortho* to the hydroxyl group.

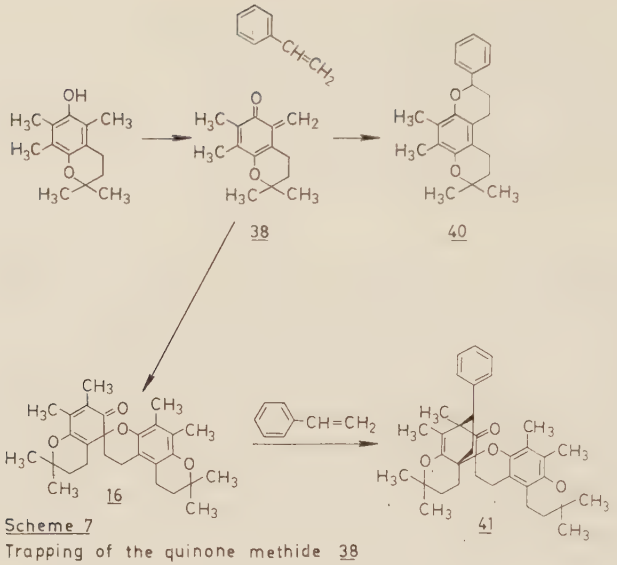
Oxidation mechanisms

Two mechanisms for conversion of tocol models with a 5-methyl group to spiro dimers can be formulated as shown in Scheme 6 for α-tocopherol. The initial reaction is formation of a phenoxy radical (*37*) [31] which can be further oxidized to a quinone methide (*38*). This is then dimerized in a Diels-Alder type reaction to the spiro dimer *16*. The radical *37* can also undergo isomerization to the benzylic radical *39* which, after coupling, gives the dihydroxy dimer *15*. This is then further oxidized and the spiro dimer *16* is formed by intramolecular radical coupling. This latter mechanism was the most likely according to the literature, and it was accordingly formulated as in III.



Scheme 6
Mechanism for formation of the spiro dimer 16.

We considered it worthwhile to carry out experiments to distinguish between the two reaction routes in Scheme 6, since the quinone methide *38* from α-tocopherol has been proposed as an intermediate in various reactions [11, 23, 24, 39—41]. To realize this we oxidized the α-model with alkaline ferricyanide in the presence of freshly distilled styrene in order to trap the quinone methide *38* as a Diels-Alder adduct (Paper VIII). This measure was successful and the compound *40* (Scheme 7) was isolated and identified. We found that styrene reacted also with the spiro dimer *16* and gave the adduct *41*.



Scheme 7
Trapping of the quinone methide 38

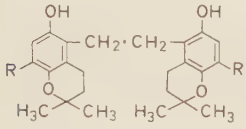
The formation of compound *40* does not rule out formation of *16* also by the alternative pathway *via* the dihydroxydimer *15*. To obtain an indication of the relative importance of the two routes, we compared the rate of formation of the spiro dimer *16* from the α-model (*22*), and from the dimer *15* (VIII). We found that formation of the spiro dimer *16* from the α-model was complete in 25 sec. or less while conversion of *15* to the spiro dimer *16* under identical conditions was complete only after 4—5 min. This indicates that the route *via* the quinone methide is at least ten times faster than that *via* the dihydroxydimer *15*, and that formation of the spiro dimer *16 via* the quinone methide *38* is the main reaction pathway.

Oxidation with *p*-benzoquinone

p-Benzoquinone was the oxidizing agent used for the synthesis of the tocopherol dimers isolated from corn oil (II and III) and for the study of tocopherol dimerization (III). In Paper IX, this preliminary investigation has been extended to all the tocol models *22*—*29*, and it has for the first time been possible to show that phenolic coupling between two molecules of the δ-model (*26*) or of the tocol model (*29*) takes place only at position 5. This is of importance since both *26* and *29* have two unsubstituted positions *ortho* to the hydroxyl group that could participate in the coupling reactions.

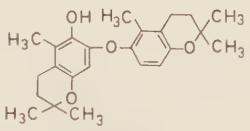
The reactions were carried out at room temperature to diminish the interconversion reactions similar to those that occur between the α-model and its oxidation products.

The tocol models with a 5-methyl group (*22*, *23*, *25* and *28*) all gave compounds formed by reactions involving the 5-methyl group. Thus oxidation of the β-model (*23*) yielded the trimer *17 b* as the main product together with the spiro dimer *30 a* and the β-tocopherylethane model *42 a*. Similarly, the spiro dimer *30 b* was isolated from oxidation of the 5,7-dimethyltocol model (*25*). 5-Methyltocol model (*28*) was not oxidized by benzoquinone and it was therefore treated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). Two products, *42 b* and *43*, were isolated. Compound *42 b* is analogous to the dimer *42 a* from the β-model and is formed by a reaction involving the 5-methyl group.



42a R = CH_3

42b R = H



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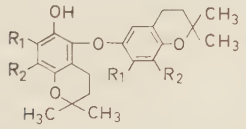
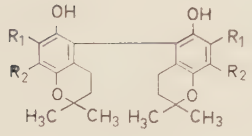
When the tocol derivatives unsubstituted at position 5 (*24*, *26*, *27* and *29*) were oxidized with benzoquinone, two types of dimers were isolated. The main products were always formed by carbon-oxygen coupling at position 5 (*44*—*47*) and the minor products were formed by carbon-carbon coupling at the same position (*48*—*50*). According to Musso [42], dimeric ethers are formed only as minor products in most oxidations and are most often observed as products of intramolecular coupling reactions. In contrast to this, benzoquinone seems to be an oxidizing agent that favours dimeric ether formation.



	R_1	R_2	Oxidation product from
44	CH_3	CH_3	γ-model (24)
45	H	CH_3	δ-model (26)
46	CH_3	H	7-methyltocol model (27)
47	H	H	tocol model (29)



	R_1	R_2	Oxidation product from
48	CH_3	CH_3	γ-model (24)
49	CH_3	H	7-methyltocol model (27)
50	H	H	tocol model (29)

Benzoquinone oxidation of tocol models unsubstituted at position 5 gives apparently much simpler products than those obtained by oxidation with alkaline ferricyanide. This permitted an unambiguous structure elucidation of all the oxidation products which revealed that all the dimers had been formed by coupling at the 5-position only, even when the

tocol derivatives have two unsubstituted positions *ortho* to the hydroxyl group as in *26* and *29*. The result thus demonstrates the strong preference for reaction at the 5-position.

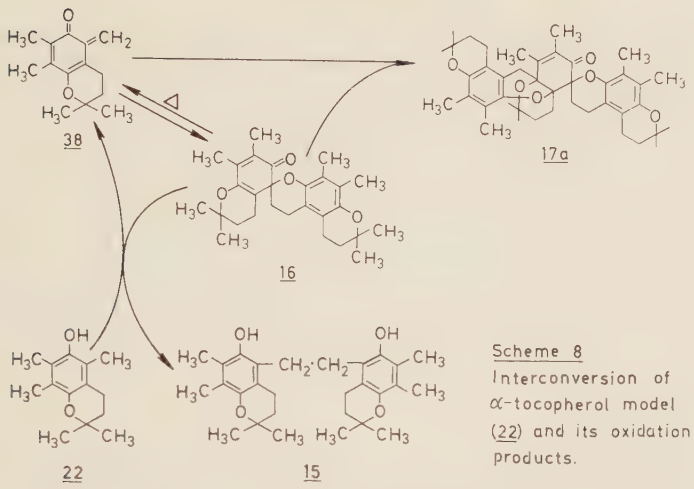
Interconversion reactions

In the oxidation of the β-model (*23*) with benzoquinone different products are obtained in refluxing benzene (III) and at room temperature (IX). When the reaction mixture was heated (III), the trimer *17 b* was the main product together with two dimers, *18* and *19*, formed as minor products by coupling at position 7. When the oxidation was carried out at room temperature (IX) the trimer *17 b* was still the main product, but the spiro dimer *30 a* and the β-tocopherylethane model *42 a* were also isolated. This indicates that the reaction temperature influences to a certain extent the course of the reaction. This was further illustrated by the study of the interconversion of α-tocopherol and its oxidation products, described in Paper X.

Oxidation of α-tocopherol with benzoquinone gives three products (III) (*15, 16* and *17 a*) while oxidation with alkaline ferricyanide gave almost exclusively compound *16* [25, 26]. Reports by Skinner and Alaupovic [25] indicated that interconversion of α-tocopherol and its oxidation products could occur, and we believed that such conversion could be more extensive in the one-phase system used in the benzoquinone oxidations than in the two-phase system used in the alkaline ferricyanide oxidations. In this latter system, the reactions giving the spiro dimer are very rapid (VIII) and the intermediate quinone methide *38* is probably not available for formation of the trimer *17 a*. (See discussion in Paper VIII).

To determine the influence of heat (X), the α-model (*22*) and the three oxidation products *15, 16* and *17* a were heated separately in benzene and the reaction mixtures were analysed by TLC. Under the conditions used, 5 % of the spiro dimer *16* was converted to the trimer *17 a*, while the other compounds were unaffected. Formation of the trimer from the spiro dimer was probably due to a retro Diels-Alder reaction of the spiro dimer to the quinone methide *38* which then reacted with another spiro dimer molecule to give the trimer.

To study the interconversion, all possible binary mixtures of the α-model (*22*) and its oxidation products *15, 16* and *17 a* were heated in benzene and the mixtures were analysed by TLC. No interconversion between the α-model and tocopherylethane *15* or between the α-model and the trimer *17 a* could be detected. However, the spiro dimer *16* and the α-model reacted with each other to form the two oxidation products *15* and *17 a*. ^{14}C-labelling of the α-model or of the spiro dimer was used to trace the origin of the reaction products. The results, depicted in Scheme 8, show that the spiro dimer *16* can oxidize the α-model to the quinone methide *38*, and *16* is concomitantly converted to the tocopherylethane *15*. The formed quinone methide can react in two ways, either with one molecule of the spiro dimer to form the trimer *17 a* or with another



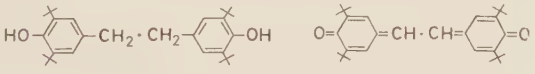
Scheme 8
Interconversion of α-tocopherol model (22) and its oxidation products.

quinone methide molecule to form the spiro dimer *via* a Diels-Alder type reaction. That this reaction is reversible under the conditions used was apparent from the radioactive experiments, as well from the results obtained when the spiro dimer was heated alone.

General discussion

The natural antioxidants, α-, β-, γ- and δ-tocopherol (*2*—*5*) are formally very similar. They all are 6-chromanols with an isoprenoid side chain in position 2 and they differ only by the number and positions of the aromatic methyl groups. It would consequently be expected that the oxidation products from the tocopherols should be very similar. However, as demonstrated in this survey, there exists considerable differences between different 6-chromanols in the case of oxidation and in the type of products formed.

Abundant literature data [42—44] indicate that the initial step in phenolic oxidation is formation of a mesomeric phenoxy radical, which then can undergo coupling reactions. In the recent review by Musso [42] on phenol coupling, this author states that dimeric and polymeric oxidation products of phenols are formed by carbon-carbon or carbon-oxygen coupling, *exclusively* at *ortho* or *para* positions to the hydroxyl group. When all the *ortho* or *para* positions are substituted with inert or bulky groups, the free phenoxy radical is comparatively stable. Benzylic coupling can occur in such cases as illustrated by the oxidation of 2,6-di-*t*-butyl-4-methylphenol, where products like *51* and *52* are observed.

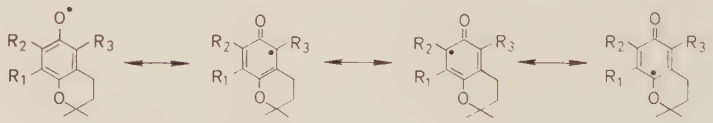


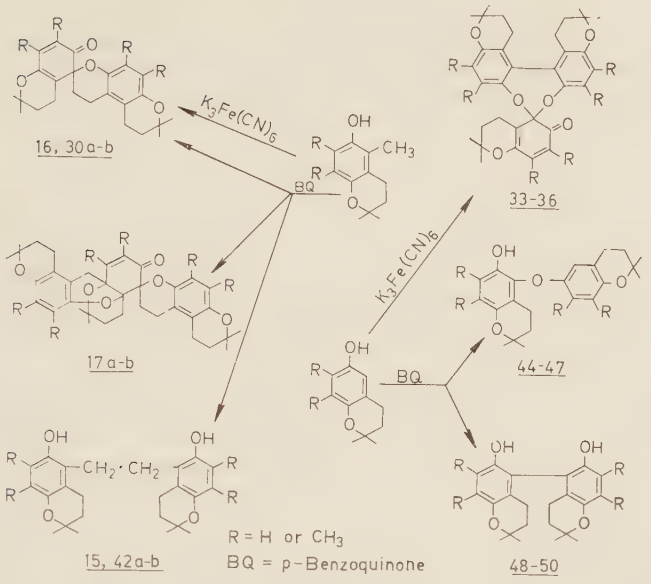
It has been clearly demonstrated in this survey that all the 6-chromanols studied have a strong preference for oxidative coupling at position 5 of the chroman moiety or at the 5-methyl group. When the 5-position is unsubstituted as in *24*, *26*, *27* and *29*, this is the sole site of reaction even if the compound has another unsubstituted position *ortho* to the hydroxyl group as in *26* and *29*, and regardless of methyl substitution at positions 7 and 8. Oxidation of other alkyl substituted *p*-alkoxyphenols gives coupling products *para* to the alkyl group [37, 48]. Analogous coupling of the tocol model (*29*) would occur at position 7, which also appears to be the less sterically hindered one. The observation that coupling occurs at position 5 only must be interpeted as a directing effect exerted by the heterocyclic ring on the aromatic one. Similar selectivity is also observed in electrophilic substitutions [49, 50]. Thus, bromination of the δ-model (*26*) yields the 5-bromo and the 7-bromo derivatives in the ratio of about 18: 1 [51].

6-Chromanols with a methyl group in position 5 (*22*, *23*, *25* and *28*) form coupling products preferentially *via* this methyl group. If the 7-position is unsubstituted (*23*, *28*) coupling occurs also there but only to a minor extent, apparently depending on the reaction temperature. β-Tocopherol (*23*) and 5-methyltocol (*28*) models appear to be the first reported examples of phenols with a free *ortho* position forming oxidation products preferentially *via* benzylic coupling.

The results described here also show that the type of adducts formed when tocol derivatives are oxidized depend on several factors, among them being substitution at position 5 in the chromanol, the oxidizing agent used and the reaction temperature. The outcome may also be affected if a one- or two-phase system is used for the oxidation (VIII). It is apparent that dimers and trimers formed by reactions involving an aromatic methyl group *e. g.* (*15*, *16* and *17*) appear only after oxidation of tocol derivatives with a methyl group in position 5. If the 5-position is unsubstituted, the adducts are formed by carbon-oxygen or carbon-carbon coupling at that ring position (VII and IX). Alkaline ferricyanide and benzoquinone both gave the same products from tocol models with a 5-methyl group while those unsubstituted at position 5 gave spiroketal trimers (*33*—*36*) with alkaline ferricyanide and simple dimers (*44*—*50*) with benzoquinone. The reactions are summarized in Scheme 9. The reaction temperature was found to influence the extent of coupling at position 7 (III and IX) as well as the interconversion of the tocol model and its oxidation products (X).

The mesomeric radical formed in the oxidation of a 6-chromanol has the form *53 a* *53 d* and coupling products would most easily arise from





Scheme 9
Main reaction pathways on oxidation of tocol models with and without a methyl group in position 5.

53 a—c. When $R_2 = R_3 = H$, coupling occurs exclusively at position 5 *via 53 b* and when $R_2 = H$ and $R_3 = CH_3$, most reactions take place at the 5-methyl group and very little coupling occurs at position 7. These experimental results indicate that *53 b* is the dominating mesomeric form of the radical and that the density of the unpaired electron at position 7 is very low.

The mesomeric radical *53 b* does not give coupling products if R_3 is a methyl group, probably because of steric hindrance. Instead it is further oxidized to the quinone methide *38* which then undergoes Diels-Alder type reactions to dimers and trimers (Scheme 6). A similar process to give a quinone methide involving the 7-methyl group does not occur, probably because *53 c* is a minor mesomeric form as indicated by the experimental results.

Phenomena analogous to the enhanced reactivity of the 5-position in 6-chromanols have previously been observed, *e. g.* in electrophilic substitutions of 5-hydroxyindane and 6-hydroxytetralin [52] and have been referred to as the Mills-Nixon effect. Recent investigations [53, 54] in this field indicate that the fusion of a small saturated ring (6-membered or smaller) to the benzene molecule causes a distortion of the geometry of the aromatic ring and thus induces a strain in the system. This effect may favour reaction at one position over a formally equivalent one, *e. g.*

by stabilizing a certain transition state. Other factors may also be involved, as indicated by our recent finding that the directing effect of the fused saturated ring is much stronger in 6-chromanol and 5-hydroxybenzofuran than in the corresponding tetralin and indane derivatives [55].

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